Molecular Aspects of the Mammalian Cell Cycle and Cancer

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ABSTRACT

Cancer arises mainly from mutations in somatic cells. However, it is not the result of a single mutation, rather, it results from increasing genetic disarray accumulated over time. Tumorigenesis in humans is, therefore, a multistep and age-dependent process. The multiple mechanisms and multiple players involved in this process necessitate an understanding of the molecular mechanisms, in order to distinctively classify the tumor sample and to assess the risk and treatment of the disease. The Oncologist 2002;7:73-81

INTRODUCTION

Cancer arises mainly from mutations in somatic cells. However, it is not the result of a single mutation, rather, it results from increasing genetic disarray accumulated over time. Tumorigenesis in humans is, therefore, a multistep and age-dependent process [1].

The multiple mechanisms and multiple players involved in this process necessitate an understanding of the molecular mechanisms, in order to distinctively classify the tumor sample and to assess the risk and treatment of the disease.

Uncontrolled cell proliferation is one of the main hallmarks of cancer, and tumor cells have acquired damage to genes that are directly involved in regulating the cell cycle [2]. Damage is caused by mutations producing an oncogene with a dominant gain of function, and/or by mutations in tumor suppressor genes causing a recessive loss of function [3, 4]. Regardless of the genetic damage or type of cancer, the common feature is a disrupted cell cycle.

In this report, the molecular mechanisms of the cell cycle are reviewed and those mechanisms known to be involved in the disruption of the cell cycle and tumor formation are discussed with an emphasis on the G1/S transition phase.

THE CELL CYCLE: REGULATING A DELICATE BALANCE BETWEEN LIFE AND DEATH

The cell cycling process is carefully regulated and responds to the specific needs of a certain tissue or cell type. Normally, in adult tissue, there is a delicate balance between cell death (programmed cell death or apoptosis) and proliferation (cell division) producing a steady state. Disruption of this equilibrium by loss of cell cycle control may eventually lead to tumor development [2].

The highly organized and regulated cell cycle process is responsible for duplication of the cell. Tight regulation and timing ensure that DNA is replicated once during the S phase (without errors), and that identical chromosomes are equally delivered to daughter cells during the M phase [2, 5]. The cell cycle is, therefore, an alteration of two main processes: A) the “doubling” process (S = synthesis phase) where DNA is synthesized, and B) the “halving” process (M = mitosis phase) where the cell and its contents are divided equally into two daughter cells (Fig. 1). The periods between these processes are called gap periods (G phase). Taken together, the cell cycle consists of the different phases listed in Table 1 [6].

CHECKPOINTS

The switch, or transition, between phases is a hallmark of the cell cycle, with an extremely accurate timing and order of molecular events. However, if something goes wrong, the cell has several systems for interrupting the cell cycle. These are the quality control points of the cell cycle and are often referred to as checkpoints [7]. At checkpoints, there are important mechanisms sensing damaged DNA before the cell enters the S phase (G1 checkpoint) or the

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**Figure 1. Cell cycle phases.**

M phase (G2 checkpoint) (Fig. 1). One major molecular hallmark of checkpoint control is where transitions turn off the previous state and promote the future state of the cell cycle (irreversible progression). Loss of checkpoint control results in genomic instability, accumulation of DNA damage, uncontrolled cell proliferation, and, eventually, tumorigenesis. Indeed, this has been implicated in the progression of many human cancers [7, 8].

**BIOCHEMICAL REGULATORY EVENTS CONTROLLING TRANSITIONS IN THE CELL CYCLE**

There are two main classes of regulatory mechanisms controlling, or driving, the cell cycle: A) the intrinsic mechanisms appearing every cycle, and B) an extrinsic mechanism, which only acts when defects are detected. The biochemical events during regulation of the components or mediators involved in these mechanisms are similar and can broadly be divided into: A) phosphorylation; B) dephosphorylation, and C) proteolytic degradation [9].

**The Main Intrinsic Actors of the Mammalian Cell Cycle**

The link between failure in checkpoint control and DNA instability was first evident in studies from the yeast *Saccharomyces cerevisiae*. The first cell cycle regulators (cdcs) were isolated and cloned from this organism, and temperature-sensitive cdc mutants from yeast have been valuable models for identification and isolation of mammalian homologues [10].

**Cyclin-Dependent Kinases (Cdk)**

Cdk's that are required for cell cycle regulation consist of an active kinase subunit in complex with a regulatory subunit, or activator, commonly called cyclin. The Cdk/cyclin complex is subjected to several kinds of regulation, both positive and negative, for instance, by reversible protein phosphorylation. Phosphorylation at specific threonine residues by the Cdk activator kinase (CAK) and dephosphorylation at specific tyrosine residues by specific Cdk phosphatases render the Cdk active (Fig. 2) [7, 11]. At least nine different Cdk's are known today, however, only some of them seem to be involved in cell cycle regulation (Table 2).

**Table 1. Cell cycle phases**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Growth and preparation of the chromosomes for replication</td>
</tr>
<tr>
<td>S</td>
<td>Synthesis of DNA (centrioles/DNA replication)</td>
</tr>
<tr>
<td>G2</td>
<td>Preparation for mitosis</td>
</tr>
<tr>
<td>M</td>
<td>Mitosis</td>
</tr>
<tr>
<td>G0</td>
<td>Temporary or permanent state of cell cycle exit. Postmitotic/terminally differentiated</td>
</tr>
</tbody>
</table>

*Exit from the cell cycle at G1, not occurring in every cell cycle.*

**Cyclins**

Cyclins are the second part of the Cdk holoenzyme and account for both substrate specificity and cell phase specificity of the Cdk/cyclin complex (Fig. 2). The cyclins are important mediators of Cdk activity, and their level fluctuates throughout the cell cycle, some being more abundant in specific cell phases than others [2]. Broadly, these cyclins have been divided into three classes: G1-S cyclins, S cyclins, and M cyclins (Table 3). The cyclin level is primarily regulated by gene expression (transcriptional regulation) and protein degradation (proteolytic regulation, ubiquitination) [12]. Cyclins respond to mitogenic signals, and unscheduled expression, leading to uncontrolled proliferation, has been implicated in different human cancers [8], for instance, in colon cancer [13] and in several incidences of breast cancer [14] (see below).
Inhibitors of Cdk

Protein inhibitors negatively regulate Cdns by direct interactions (Fig. 2). Cdk inhibitors (CKI) are divided into two families according to substrate specificity. In mammalian cells, these are the Cip/Kip family, consisting of p21Cip/Kip, p27Cip/Kip, and p57Cip/Kip, and the INK4a family, including p15INK4a, p16INK4a, and p18INK4a, the latter being potent inhibitors of Cdk4/Cdk6/CyclinD complex (see below) [15]. CKIs mediate cell cycle arrest in response to several antiproliferative signals. Disturbed CKI action has been identified in different human cancers [16], suggesting a role for CKIs in preventing uncontrolled proliferation and tumor formation (see below).

Tumor Suppressor Genes

Tumor suppressors are main effectors of the cell cycle clock; some of them are extrinsic factors in that they only act if the cell is damaged. The most important, in respect to cancer, are the well-known Retinoblastoma (Rb) protein and the transcription factor p53.

Rb

Rb is a juvenile eye cancer that is caused by a mutation in the Rb gene, located on human chromosome 13. The main function of Rb is to connect the cell cycle clock to the transcriptional machinery (intrinsic mechanism). The Rb protein interacts with a protein called E2F, which is a nuclear transcription factor involved in cellular replication during the S phase. Interaction between Rb and E2F prevents E2F from functioning as a transcription factor. However, Rb is only able to bind E2F when it is unphosphorylated. It will not interact with E2F in its hyperphosphorylated state (Fig. 3). Rb mutants, which are constitutively phosphorylated and cannot bind E2F, provide uncontrolled cell division at the S-phase restriction site and cells may become tumorigenic. In a subset of human cancers, growth advantage has been accomplished by direct mutation and/or loss of function of Rb [17, 18]. The “Rb pathway” is further discussed in view of the G1-S transition phase below.

p53

Another protein critical in regulating the cell cycle is the tumor suppressor protein p53. p53 is a DNA-binding protein regulating the expression of genes involved in cell cycle arrest. It senses DNA damage and tells the cell to either stop growing (until damage is repaired) or to kill itself by apoptosis (preventing unregulated cellular growth and formation of cancer), a typical extrinsic mechanism. p53 is the most frequently disrupted gene in human cancers. In fact, more than 50% of human cancers are associated with a p53 mutation, including cancers of the bladder, breast, cervix, colon, lung, liver, prostate, and skin. p53-related cancers are also very aggressive and have a high degree of lethality [6].

Restriction Point Control: The G1-S Transition

The G1-S transition is a highly regulated and important transition in the cell cycle. At this stage, the cell cycle passes a point between the G1 and S phase (restriction point) with an

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**Table 2. Cdns involved in yeast and mammalian cell cycles**

<table>
<thead>
<tr>
<th>Species</th>
<th>Name</th>
<th>Original name</th>
<th>Size (aa)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td>Cdk1</td>
<td>Cdc28</td>
<td>298</td>
<td>All phases</td>
</tr>
<tr>
<td>S. pombe</td>
<td>Cdk1</td>
<td>Cck2</td>
<td>297</td>
<td>All phases</td>
</tr>
<tr>
<td>H. sapiens</td>
<td>Cdk1</td>
<td>Cdc2</td>
<td>297</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Cdk2</td>
<td></td>
<td>298</td>
<td>G1, S, S (M?)</td>
</tr>
<tr>
<td></td>
<td>Cdk4</td>
<td></td>
<td>303</td>
<td>G1</td>
</tr>
<tr>
<td></td>
<td>Cdk6</td>
<td></td>
<td>326</td>
<td>G1</td>
</tr>
</tbody>
</table>

**Table 3. Major cyclin classes involved in cell cycle control**

<table>
<thead>
<tr>
<th>Species</th>
<th>Class</th>
<th>G1/S</th>
<th>S</th>
<th>M</th>
<th>G1</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td>Cln 1, 2</td>
<td>Clb 5, 6</td>
<td>Clb 1, 2, 3, 4</td>
<td>Clb 3</td>
<td></td>
</tr>
<tr>
<td>S. pombe</td>
<td>Puc1</td>
<td>Cig 2</td>
<td>Cdc13</td>
<td>Puc1</td>
<td></td>
</tr>
<tr>
<td>H. sapiens</td>
<td>Cyclin E</td>
<td>Cyclin A1, 2</td>
<td>Cyclin B1, 2</td>
<td>Cyclin D1, 2, 3</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 2. Regulation of Cdk activity.** Cdk activity is regulated by reversible phosphorylation, by activators or inhibitors.
irreversible commitment to a new cycle. Stimulation of cells with mitogens results in cell cycle progression. The underlying molecular mechanisms are the induced expression of Cdks and cyclins required for the cell to progress from the early G₁ phase into the late G₁ phase of the cell cycle, reaching the restriction point. This is a critical point late in the G₁ phase where the mammalian cell becomes committed to entering the S phase and to completing the cell cycle, even in the absence of growth factors [19, 20]. The main Cdks involved in progression from mid- to late G₁ are Cdk4 and Cdk6, driven by three G₁-specific cyclins, D1, D2 and D3. The main cellular events regulating the onset of the Rb pathway are phosphorylation and inactivation of Rb (Figs. 3 and 4). The Cdk2/Cyclin E complex has a secondary role in Rb phosphorylation. Cyclin E transcription is activated when Rb is already hyperphosphorylated by Cdk4/Cyclin D and no longer can repress the transcription driven by E2F. In this way, the formation of Cdk2/Cyclin E complex in the late G₁ phase acts as a positive feedback loop on the Rb pathway already initiated by Cdk4/Cyclin D (Fig. 4) [20].

Regulation of G₁ Progression

G₁ progression requires sustained Cyclin D expression, persisting as long as there are ongoing mitogenic signals. Mitogenic transcription activation of D-type cyclins has been shown to be induced, for instance, by c-Myc, AP-1, and NF-kB transcription factors [9] (Fig. 5A). However, when mitogens are removed, the level of Cyclin D rapidly decreases and the cell is arrested in G₁. A similar block in Cyclin D-kinase activity and subsequent arrest in G₁ phase is achieved by the INK4a family of CKIs (Fig. 5A) [2]. Four different members of this family (p16INK4a, p15INK4a, p18INK4a, and p19INK4a) are known to bind and inhibit Cdk4 and Cdk6, without affecting other Cdks [2]. The INK4a-bound Cdks are not able to complex with cyclin, and stay intact as an INK-Cdk heterodimer. In contrast, the Cip/Kip family of CKIs (p27Cip/Kip, p21Cip/Kip, and p57Cip/Kip), which do not inhibit the G₁ phase, play a positive role by stabilizing the Cdk/cyclin complex (Fig. 5A) [3, 15]. Regulation of INK4a appears to be at the level of transcription, in response to transforming growth factor-β (TGF-β) [21], and coupled with degradation by ubiquitin-mediated proteolysis (see detailed description below). Thus, only p19INK4a seems to be periodic throughout the cell cycle [22].

Cyclins are unstable proteins and their levels vary throughout the cell cycle. This is due to degradation by the ubiquitin/proteasome pathway when they are not required (Fig. 5A) [1, 4]. The ubiquitin/proteasome pathway is an important ATP-dependent degradation pathway controlling the irreversible destruction of regulatory proteins. This process relies on the sequential action of the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2, and the ubiquitin-ligase E3 [23]. Distinct ubiquitination pathways operate on the G₁ cyclins (D1, D2, D3, and E) depending on whether or not the cyclin is bound to Cdk. Degradation of Cyclin D is dependent on phosphorylation when bound to Cdk4 and independent of phosphorylation when unbound (Fig. 5A) [1, 4, 24].

Cdk activity is inhibited by phosphorylation on specific tyrosine residues, and phosphatase treatment leads to a hyperactive kinase (Fig. 5A) [5]. Three different mammalian phosphatases are known, Cdc25 A, B, and C. The regulation of Cdc25A is critical for G₁ response to DNA damage. Cells respond to UV irradiation by rapid proteolytic degradation of Cdc25A via p53-dependent accumulation of p21Cip/Kip CKI, which is essential for DNA repair and survival (Fig. 5A)
Figure 5. Molecular mechanisms regulating the G₁-S transition.
In G1-arrested cells, Cdk4 is phosphorylated at tyrosine 17, and UV irradiation prevents dephosphorylation and re-entry into the cell cycle, suggesting that Cdk4 is a target for Cdc25A [26]. To conclude, the main role of the Cdk4/6/Cyclin D complex in the early progression of G1 is to phosphorylate Rb and other negative regulators of the cell cycle, and thereby promote cell cycle progression (Fig. 5A) [7]. This process is tightly regulated as summarized in Figure 5A and reviewed in [9].

Rb phosphorylation releases E2F and allows the expression of regulators required for DNA synthesis and S phase progression (Fig. 3). E2F triggers expression of proteins like dihydrofolate reductase, thymidine kinase, different DNA polymerases and the late-G1 cyclin E (Fig. 4B) [27]. Expression of Cyclin E establishes a positive feedback loop of Rb phosphorylation, since Cyclin E in complex with Cdk2 will continue to phosphorylate Rb (Fig. 5B) [4], contributing to an irreversible transition into the S phase and cell cycle progression, even in the absence of growth factors [28]. Negative regulation of Cyclin E is also mediated by ubiquitin/proteolytic degradation, however, as far as is known, only when it is phosphorylated on a specific threonine residue and in complex with Cdk2 [29] (Fig. 5B) [1] (in contrast to Cyclin D). Inhibitors of Cdk2/Cyclin E are members of the Cip/Kip family of CKIs, such as p27Kip1 and p21Kip1, which are transcriptionally activated by p53 and is largely responsible for the p53-dependent G1 arrest in response to stress and DNA damage.

This is due to the accumulation of p21Kip1 followed by inhibition of Cdk2/Cyclin E and a block in the progression from the G1 into the S phase (Fig. 5B) [3]. Similar to Cdk4, Cdk2 is also a target for Cdc25 phosphatase (Fig. 5B) [2]. Cdc25A is active in the late G1 phase, corresponding to the time when Cdk2/Cyclin E is acting, suggesting that the activation is due to dephosphorylation by Cdc25A [30]. In conclusion, the mechanism of regulation in late-G1 transition (of Cdk2/Cyclin E) is similar in many ways to the regulation in early-G1 transition (of Cdk4/6/Cyclin D) (compare Figs. 5A and 5B). The biochemical events are primarily phosphorylation, dephosphorylation, and ubiquitination, with the overall mission to either prevent or induce a new cell cycle via the Rb pathway.

**Disruption in the G1-S Transition and Cancer**

**Rb Pathway Disruption**

The cell cycle regulatory genes most often altered in tumors are those involved in controlling the G1-S transition through the regulation of the Rb pathway (described above). The overall mechanism of tumor formation seems to consist of inhibitory effects on the Rb pathway, resulting in a growth advantage [2]. This is accomplished in many different human tumors by different gain-of-function mutations (proto-oncogenes) and/or loss-of-function mutations (tumor suppressor genes). Rb is mutated in several human tumors [17, 18]. Mutated or deleted Rb is no longer able to repress the function of E2F (Fig. 3). Rb normally protects against cancer development in its dominant phenotype. Therefore, both alleles must be mutant for the disease to develop (homozygous). Loss of heterozygosity is a classic feature of tumor suppressor genes. The Rb gene is implicated most often in adult cancers, particularly small cell carcinomas, and inherited allelic loss of Rb confers increased susceptibility to cancer formation [2]. An inactivation of the Rb pathway is also accomplished by mutations in other regulatory components. Loss-of-function mutations in the INK4a family members, particularly p16INK4a, occur frequently in human cancers [2, 31]. In familial melanomas, for instance, one copy of mutated p16INK4a is inherited and the second is lost in the tumor cell (loss of heterozygosity). These mutations are found in over 50% of all familial melanomas. Homozygous deletions of the INK4a locus are a common feature in gliomas, mesotheliomas, carcinomas, acute lymphocytic leukemias, sarcomas, ovarian cancer, and probably also others. Reciprocally, mutations in Cdk4 resulting in lost ability to bind p16INK4a, have been found in some melanomas [2].

Gain-of-function mutations are also involved in disrupting the Rb pathway by overexpression of cyclins. For instance, the accumulation of Cyclin D1 is found to be implicated in most human colon cancers. Colorectal cancer is a classic example of the hallmarks of tumor formation; that cancer is a “disease of age”, resulting from an accumulation of sequential mutations in tumor suppressor genes and proto-oncogenes that eventually lead to tumor formation [1]. Recently, it has been shown that mutation in the APC (adenomatous polyposis coli) tumor suppressor gene results in an accumulation of β-catenin transcription factor, common in colon cancers, and leads to increased and unscheduled Cyclin D1 expression [13]. In addition, there is evidence for the participation of the G1 cyclins (D and E) in breast cancer. Overexpression of Cyclin D1 has been reported in ductal carcinoma in situ, and similar overexpression of Cyclin E has been suggested [32]. Cyclin D1 is also overexpressed in over 50% of mammary carcinomas [33]. Recently, it has been shown that there is an absolute requirement for Cyclin D1 overexpression in malignancy transformation that cannot be complemented by other, closely related cyclins like D2 and D3. This supports putative anti-Cyclin D therapy highly specific for breast cancer [14]. Taken together, the overall mechanisms of disturbing the Rb pathway converge into one common scheme: the liberation of E2F from Rb control and the progression of cells from G1.
into S phase become uncontrolled. The cell no longer responds to antiproliferative signals normally working in this phase of the cell cycle, and it is no longer dependent on growth factors like TGF-β [1].

The loss of function in INK4a mimics Cdk hyperactivity and overexpression of cyclin, which all lead to Rb hyperphosphorylation and disruption of the G1-S restriction point. This supports the observation that inactivation of one of these components in the RB pathway results in decreased tumor suppression [34]. Hence, the disruption of the p16\textsuperscript{INK4a}-Cyclin D/Cdk4-Rb pathway seems to be a common part of the life history of human cancers, independent of patient age or tumor type. Other G1-S regulators, like E2F, Cyclin E, and the Cip/Kip family members of CKIs, are rarely lost or mutated in human cancers.

**p53 Pathway Disruption**

Although the Rb pathway is the underlying mechanism in the G1-S transition, the tumor suppressor p53 is also an important regulator of the G1-S cell cycle checkpoint. p53, unlike the other regulators in the Rb pathway, is not required for cell cycle progression. Hence, its role is to break the cycle only when the cell is damaged, by either G1 arrest or by inducing cell suicide (apoptosis) [35]. Due to the fact that p53 is the most frequently mutated gene in human cancers, it is a crucial target for therapy in respect to tumor formation and elimination of damaged cells. p53 is short-lived and activated in response to UV irradiation, DNA damage, cellular stress, etc. [2]. The CKI p21\textsuperscript{Cip/Kip} is transcriptionally activated by p53 and is required for p53-mediated G1 arrest. However, p21\textsuperscript{Cip/Kip} is not required for p53-mediated apoptosis. As previously described, p21\textsuperscript{Cip/Kip} inhibits the Rb pathway (Fig. 5B).

With the loss of Rb pathway function, the cell is, therefore, able to bypass the G1 arrest mediated by p53. Thus, loss-of-function mutations in p53 mimic the loss of the Rb pathway in respect to deregulated G1-S transition, and the cell becomes tumorigenic. p53 transcriptionally activates its own inhibitor, Mdm2, ensuring a negative feedback regulation. Mdm2 inhibits p53 transcription, targets p53 for degradation by the ubiquitin/proteasome pathway, and enforces the transport of p53 into the cytoplasm, where it is degraded [8]. ARF is expressed upon abnormal mitogenic signaling by overexpressed transcription factors (oncoproteins) like E2F, Ras, c-Myc, and vAbl. In this way, ARF connects the Rb and Mdm2-p53 pathways. There are reasons to believe that ARF reduces the ability of p53 to be a tumor suppressor. Disruption of the ARF-Mdm2-p53 pathway is frequently found in many human cancers, and it seems to be a common part of cancer life history, independent of age or tumor type, similar to disruption in the p16-Cyclin D/Cdk4-Rb pathway [8]. Recently, it has been shown that both p16\textsuperscript{INK4a} and p19\textsuperscript{ARF} are acting as strong tumor suppressors in mice models, and that double-acting mutations (knockout mice) of p16\textsuperscript{INK4a} and p19\textsuperscript{ARF} are required for severe cancer formation [37].

**Prognostic and Therapeutic Aspects**

The enormous progress in understanding the molecular mechanisms of the mammalian cell cycle and its involvement in cancer development in the last decades has shown that cell cycle regulators have a huge potential both as prognostic and therapeutic markers of cancer. Genetic alterations, implicated in disturbed regulation of the G1-S transition (discussed above), provide relevant information to assess the risk or prognosis of the disease and target therapy [38].

Recently, it has been shown that p16\textsuperscript{INK4a}-status has an important prognostic relevance for patients with pancreatic cancer, where alterations in p16\textsuperscript{INK4a} are connected with a bad prognosis [39]. Evaluation of the prognostic significance of p21\textsuperscript{Cip/Kip} and p53 in patients with gallbladder carcinomas showed that reduced expression of p21\textsuperscript{Cip/Kip} and overexpression of p53 were associated with a shortened disease-free stage [40].

The molecular defects in G1-S regulators in a given cancer affect the outcome of radiotherapy or chemotherapy treatment. For instance, the efficiency of radiotherapy-induced p53 apoptosis or cell cycle arrest will not be optimal in tumors with deleted or mutated p53.

Increased knowledge of the molecular mechanisms of G1-S transition involved in tumor formation suggests that modulators of Cdks and cyclins are potent therapeutic targets in cancer therapy [41, 42]. There are currently extensive efforts being made to develop new therapeutic anticancer agents specifically targeting these modulators, and several agents are currently in clinical trials [43, 44]. The specific Cdk inhibitor flavopiridol, for instance, is the first Cdk modulator tested in clinical trials (already in phase II). Flavopiridol most effectively inhibits Cdk1, Cdk2, and Cdk4. Treatment with flavopiridol has resulted in blocking cell cycle progression, promoting differentiation, and inducing apoptosis in various types of cancerous cells. Infusions of the Cdk inhibitor in patients with colon and gastric carcinomas, prostate cancer,
renal cancer, and non-Hodgkin’s lymphoma have been done successfully, although there are some minor side effects [44].

Another Cdk modulator currently being tested is UCN-10. This modulator has been shown to block the cell cycle and induce apoptosis in hematopoietic models [43] with promising results. Several other chemical Cdk inhibitors have been developed, like paullonines and indurines, showing a potential for anticancer treatment in vitro [45, 46]. However, the inability to target the drugs or genes to specific cancer cells makes therapy difficult. The main approach is to take advantage of the weakness of tumors, most of them lack Rb and/or p53, and selectively kill them. This is indeed the case in a recent study, utilizing adenovirus-associated virus which selectively infects and kills cells lacking p53 [47]. Another recent approach is the use of antisense oligonucleotides to specifically target cell cycle regulators. A recent study using Cyclin D1 antisense oligonucleotide showed cell death induction specifically induced in colon cancer cells [48].

Although great progress in understanding the molecular aspects of cancer has been made and several therapeutic agents have been developed, it is still difficult to cure cancer. Tumor formation is a multistep process, and the components of the different cell cycle phases crosstalk with each other and other components. The inactivation of Cyclin D1 for instance, resulting in a block in the Rb pathway, may have far-reaching consequences, and the Rb pathway block might be bypassed by other crosstalk components. Combined treatment using conventional chemotherapy together with new specific therapeutic agents might be a compromise. It still remains to find the correct combination for each and every incidence of cancer.

**SUMMARY**

A subset of proto-oncogenes and tumor suppressor genes has been identified to be involved in the uncoupling of the cell cycle from its normal regulation. Their signaling pathways seem to converge on the machinery involved in passing from the G1 phase into the S phase and allowing the cell to exit the cycle (the Rb and the p53 pathways) (Fig. 5). Disabling the Rb and p53 pathways is clearly a hallmark of human cancer. The ability to restore these functions is likely to be a very efficient way to treat cancer. In this respect, there have been major advances in understanding the mechanisms of cell cycle regulation, in particular the G1-S phase. The progress in understanding the molecular events of cell cycle regulation, like phosphorylation/dephosphorylation, macromolecular substrate binding, cyclins as regulatory subunits, cellular localization, proteolytic degradation, and last but not least, the increasing understanding of “crosstalk” between pathways, has been enormous and has lead to the development of many therapeutic agents, which are already in human trials. From this point of view, it is obvious that the full potential of cancer therapy as small molecule inhibitors has yet to be reached.

As a final comment, to emphasize the importance of an increased understanding of the molecular events of the cell cycle, the action of the cell cycle most likely is involved in other noncancerous diseases as well, which also need to be explained and treated.

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